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COMPARATIVE STUDY OF DIFFERENT ANTIGENS AND OF DIFFERENT TEMPERATURES OF INCUBATION IN THE WASSERMANN TEST*

J. WHEELER SMITH, JR., AND W. J. MACNEAL

From the Laboratories of the New York Post-Graduate Medical School and Hospital, New York

INTRODUCTION

It is well recognized that widely different results may be obtained in complement-fixation tests for syphilis by the use of different antigens and by the employment of different incubation conditions, even with the same antigens. In a previous communication[1] we have reported on Wassermann tests that were performed by 3 methods on 496 identical specimens from 477 patients. In the 1st method a cholesterin-reinforced antigen was employed and the 1st incubation was carried out at 37 C. In the 2nd method a simple alcoholic extract was used as antigen, with incubation also at 37 C. In the 3rd method this latter antigen was again employed, but the 1st incubation was carried out in the refrigerator for 4-24 hours. The last method proved more sensitive in the group of known syphilitics than the other procedures tested. Furthermore, a positive result thus obtained proved to be more trustworthy evidence of syphilis than did positive results obtained with the cholesterinized antigen and 1st incubation at 37 C.

In the present paper we shall present the results of Wassermann tests performed by 6 methods on 501 identical specimens from 457 patients. In the 1st of these 6 methods a cholesterin-reinforced antigen was employed, and the 1st incubation was carried out at 37 C. In the 2nd method a simple alcoholic extract was used as antigen, with incubation also at 37 C. In the 3rd method an antigen prepared from the acetone-insoluble fraction of beef heart, after the method of Noguchi,[2] was used, with incubation also at 37 C. Exact duplicates of these tests were prepared and incubated at 8 C., for 4 hours. Tests 4, 5, and 6 differ from Tests 1, 2, and 3, respectively, only in the temperature and length of time for the 1st incubation.

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[1] Smith, J. Wheeler, Jr., and MacNeal, W. J.: Jour. Immunol., 1916, 2, p. 75.

[2] Serum Diagnosis of Syphilis and the Butyric Acid Test for Syphilis, 1911, p. 79.

PREPARATION OF REAGENTS AND THE TECHNIC OF THE TESTS

The antigens used in this study were all prepared from beef heart. Fat and fibrous tissue were cut away, blood clots were washed out with cold water, and the lean muscle was wiped dry and passed through a meat-chopper. One hundred and twenty grams of the chopped meat were extracted in 1200 c.c. of absolute alcohol at 37 C., with occasional shaking, for 2 weeks. The preparation was then filtered through paper until clear. A portion of the filtrate, measuring 100 c.c., was put aside for use as simple alcoholic extract antigen, designated as B. H. P. (beef heart, plain). To another portion of 100 c.c., 0.4 gm. of cholesterin was added. This mixture was refrigerated over night and again filtered. The filtrate was employed as cholesterin-reinforced antigen, labeled B. H. C. (beef heart, cholesterinized). What remained of the original filtrate, approximately 1000 c.c., was used in the preparation of the acetone-insoluble antigen of Noguchi.[2] It was identified as Antigen B. H. A. (beef heart, acetone-insoluble fraction).

TABLE 1

SCHEME OF TITRATION OF THE ANTIGENS

(a) Hemolytic Titration

Antigen, 10% emulsion	0.1	0.2	0.3	0.4	
Salt solution, 0.9%	0.7	0.6	0.5	0.4	
R. B. C., 5% suspension	0.2	0.2	0.2	0.2	

Incubate 2 sets of tubes, one at 37 C. for 30 minutes and the other at 8 C. for 4 hours and make readings.

(b) Anticomplementary Titration

Antigen, 10% emulsion	0.1	0.2	0.3	0.4	
Salt solution, 0.9%	0.3	0.2	0.1		
Complement, 10%	0.2	0.2	0.2	0.2	

Incubate 2 sets of tubes, one at 37 C. for 1 hour and the other at 8 C. for 4 hours. Then add 0.1 c.c. of suspension of sensitized red blood cells, incubate at 37 C. for 30 minutes, and make readings.

(c) Specific Antigenic Titration

Antigen, 10% emulsion	...	...	...	...	...	0.05	0.1	0.2	
Antigen, 1% emulsion	0.01	0.02	0.05	0.1	0.2				
Very positive serum, 20%	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2
Salt solution, 0.9%	0.3	0.3	0.3	0.2	0.1	0.3	0.2	0.1	0.3
Complement, 10%	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

Incubate for fixation, one set at 37 C., for 1 hour and the other at 8 C., for 4 hours. Then add 0.4 c.c. of suspension of sensitized red blood cells, incubate at 37 C. for 30 minutes, and make readings.

Each antigen was titrated for (1) hemolytic effect, (2) anticomplementary action, and (3) specific antigenic property. The titrations were made for each set of incubation conditions, that is, 37 C. for 1 hour and 8 C. for 4 hours.

A type protocol of the titrations of the antigens is shown in Table 1.

When tested according to this scheme, the plain alcoholic extract, Antigen No. 74, B. H. P., was not hemolytic and not anticomplementary at either temperature in the maximum quantities tested. The antigenic titer showed the smallest amount necessary for complete fixation to be 0.05 c.c. of a 10% emulsion, at 37 C., and 0.2 c.c. of a 1% emulsion at 8 C. The cholesterinized alcoholic extract, Antigen 74 (a), B. H. C., was not hemolytic at either temperature in the maximum quantities tested. It was anticomplementary in dose of 0.4 c.c. at 37 C., and also at 8 C. The antigenic titer proved to be 0.2 c.c. of a 1% emulsion, at 37 C., and 0.1 c.c. of a 1% emulsion at 8 C. The corresponding acetone-insoluble preparation, Antigen No. 75, B. H. A., was not hemolytic at 37 C., nor at 8 C. in the maximum quantities tested. It was anticomplementary in dose of 0.4 c.c. at both temperatures. Its antigenic titer was 0.05 c.c. of a 1% emulsion, at 37 C., and 0.01 c.c. of 1% emulsion at 8 C.

It will be observed that the only differences in the titers occurred in the tests of the specific antigenic property and in the absence of apparent anticomplementary action from Antigen B. H. P. The preparations were all more antigenic at 8 C. than at 37 C. The titrations recorded were those made when the antigens were prepared, in September, 1916. This study was made during September, October and November of 1916 and, occasionally, toward the latter part of the work, the anticomplementary controls on Antigen B. H. C. indicated an increasing anticomplementary titer in that antigen. At such times the antigen was anticomplementary in 0.4 c.c. at 37 C., but in 0.3 c.c. at 8 C. On 1 or 2 occasions, when the hemolytic system was a bit tighter than usual, Antigen B. H. C. was found to be anticomplementary in 0.2 c.c., and partially so in 0.1 c.c. It would, therefore, seem to be advisable to employ a more dilute emulsion of the cholesterinized antigen for use at 8 C. It is quite possible to do so, for the antigenic titer, as has been noted, is higher at 8 C. than at 37 C.

The hemolytic system used was the sheep-rabbit system. The unit of cells was 0.2 c.c. of a 5% suspension of sheep's erythrocytes, repeatedly washed and finally packed for 15 minutes at 1500 r. p. m. The unit of complement was 0.1 c.c. of a 10% solution of fresh, active guinea-pig serum. The unit of amboceptor was determined each time. For this study we employed an amboceptor serum of which the unit was always in the neighborhood of 0.1 c.c. of 1:1000 dilution, that being the quantity which, with 1 unit of complement, effected complete hemolysis of 1 unit of cells in 15 minutes at 37 C. Two units each of amboceptor and complement were used in the tests. This system, it will be observed, is quite sufficiently loose to obviate very largely the danger of nonspecific fixation. And yet, as will subsequently appear, we have found reactions in 16 of the 501 tests, or 3.2%, which we feel inclined to regard as false positive reactions (Tables 10 and 11).

The serums to be tested were always heated at 56 C., for 30 minutes, just prior to use.

The tests for incubation at 37 C. and those for incubation at 8 C. were exact duplicates. In each case 4 tubes were used, the contents of which were as follows: Tube 1: patient's serum, 0.04 c.c.; Antigen B. H. C., 0.1 c.c.; complement, 0.2 c.c.; salt solution to make 0.6 c.c. Tube 2: patient's serum, 0.04 c.c.; Antigen B. H. P., 0.1 c.c.; complement, 0.2 c.c.; salt solution to balance. Tube 3: patient's serum, 0.04 c.c.; Antigen B. H. A., 0.1 c.c.; complement, 0.2 c.c.; salt solution to balance. Tube 4: patient's serum, 0.08 c.c.; no antigen; complement, 0.2 c.c.; salt solution to balance. One set of tubes was incubated at 37 C., for 1 hour; the other set at 8 C. (ice-box), for 4 hours. At the end of the period of incubation, in each case, 0.4 c.c. of sensitized red cells was added to each tube and the tubes were again incubated. Those which had been kept in the ice-box were put into a water bath, the initial temperature of which was 45 C. This was because the tubes and the contents had become so thoroughly chilled that it was necessary to employ a greater degree of heat for them than for the others, to allow for absorption. No further heat was applied during incubation, so that at the end of about 15 minutes the temperature of the bath had fallen to about 37 C. The tests were read when the control tubes showed complete hemolysis, which was usually in 15-30 minutes. A representation of the set-up is shown in Table 2.

TABLE 2

Scheme of Setting Up the Tests

	1	2	3	4
Patient's serum	0.04	0.04	0.04	0.08
Salt solution	0.3	0.3	0.3	0.3
	B. H. C.	B. H. P.	B. H. A.	
Antigen	0.1	0.1	0.1	0.0
Complement	0.2	0.2	0.2	0.2
Incubation				
Sensitized cells	0.4	0.4	0.4	0.4

RESULTS OF THE TESTS

The cases herewith presented many be divided into 2 classes on the basis of history. The known syphilitics make up the 1st class. They gave histories of infection, and most of them had had treatment or were under treatment at the time the tests were made. This class we shall call Group I, and in this group we record 92 reactions in 80 cases. This record is presented briefly in Table 3 and, in greater detail, in Table 4.

TABLE 3

Summarized Results of 92 Tests on 80 Syphilitic Patients, Group I

Antigen and Temperature	Positive		Doubtful		Negative	
	Number	Per Cent.	Number	Per Cent.	Number	Per Cent.
1. Cholesterinized at 37 C.	48	52.1	3	3.2	41	44.7
2. Simple at 37 C.	24	26.0	1	1.0	67	73.0
3. Acetone insoluble at 37 C.	29	31.5	0	0.0	63	68.5
4. Cholesterinized at 8 C.	70	76.0	3	3.2	19	20.8
5. Simple at 8 C.	59	64.1	4	4.3	29	31.6
6. Acetone insoluble at 8 C.	43	46.7	3	3.2	46	50.1

The figures presented in Table 3 demonstrate clearly the greater delicacy obtained by ice-box incubation. At 37 C., antigens B. H. C., B. H. P., and B. H. A. yielded, respectively, 52.1%, 26.0%, and 31.5% of positive reactions, while by the ice-box method, on the other hand, they gave, in the same order, 76%, 64.1%, and 46.7% positive tests.

It should be noted that the simple extract antigen at 8 C., with 64.1% positive, is much superior to the cholesterinized antigen at 37 C., which yielded 52.1% positive. This is a confirmation of 1 of the findings which we have already recorded.[1] The cholesterin-reinforced antigen at 8 C. yields the highest percentage of positive reactions in cases of syphilis. It may be noted that although Antigen B. H. A. is of slightly greater value than Antigen B. H. P. at 37 C., it is much inferior at 8 C.

The 2nd class of cases is composed of those in which there are no histories of syphilis. We shall subdivide this class by a consideration of factors other than the history. It is, of course, manifestly absurd to consider a case nonsyphilitic simply because there is no history of syphilis. It becomes necessary to consider the physical findings, the visible and other evidences of disease. On this basis it is possible to form several subdivisions of this group, the 1st of which consists of those cases which presented lesions typically luetic or which, in the absence of other etiologic factors, are usually considered to be luetic. This group we shall call Group II A. It contains cases which, although not avowedly luetic, are probably luetic. We have placed 24 cases in Group II A and on the serums and cerebro-spinal fluids of these, 27 tests have been made. The results are summarized briefly in Table 5 and presented in greater detail in Table 6.

A comparison of Tables 3 and 5 reveals a remarkable similarity. Although the figures in Table 5 are absolutely much higher than those of Table 3, due, undoubtedly, to the fact that the cases of Group II A had not received antiluetic treatment, the relations among the figures themselves are very much the same. Thus, as in Table 3, although Antigen B. H. C. is superior to Antigen B. H. P. at 37 C., the latter at 8 C. is equal to Antigen B. H. C. at 37 C., but Antigen B. H. C. at 8 C. is best of all, yielding 100% positive reactions.

There seems to be little, if any, doubt concerning the luetic nature of cases in Group II A. This cannot be said, however, of all the cases in the next group, which we shall call Group II B. They were cases with no histories of syphilis nor of antisyphilitic treatment, and in which the physical findings were rather indefinite or, as in 3 of the cases, entirely negative. In each of these cases there was, however, a note of suspicion in the history. Thus, in the case of Specimen 7, in Table 8, the patient was the sweetheart of the patient who furnished Specimen 6, in Table 4. In Case 9 the history indicated a possible

TABLE 4

Brief Data Concerning 92 Tests on 80 Syphilitic Patients, Group I

No.	History of Infection	Treatment	Sex	Age	Manifestations at Time of the Tests
1	16 years ago	....	♂	37	Mild jaundice and gastric disturbance
2	14 years ago	606 (VI); Hg (X)	♂	31	Sore throat
3	3 weeks ago	....	♂	26	Sore on penis
4	1½ years ago	606 (II); Hg; KI	♂	49	Pain in knees
5	....	606; Hg	♂	23	No manifestations
6	2 years ago	Hg (very little)	♂	21	Tumor of liver
7	....	....	♂	34	Ulceration of hard palate
8	20 years ago	....	♂	42	Old ulcer of tongue
9	7 years ago	Hg (very little)	♂	40	Leukoplakia
10*	2 years ago	Hg (very little)	♂	21	Tumor of liver
11	4 years ago	....	♀	35	Poor vision; rhinitis
12	....	606; Hg; KI	♂	39	....
13	....	....	♂	42	....
14†	....	606; Hg; KI	♂	39	....
15	....	....	♂	30	No manifestations
16	1904	606 (II); Hg	♂	35	No manifestations
17	1896	Hg; KI	♂	38	Nervousness
18	6 weeks ago	....	♂	33	Sore throat and rash for 2 weeks
19	7 months ago	606 (I); Hg (X)	♂	23	No manifestations
20	28 years ago	606: Hg	♀	48	No manifestations
21	6 years ago	Hg; KI	♂	30	Pains in legs
22	Acquired from father	606; Hg	♂	10	Optic neuritis; sore throat
23	1904	Hg	♂	38	Sores on face
24	....	....	♀	53	Nasal and pharyngeal ulceration
25	....	606 (VI); Hg (XV)	♂	24	....
26	....	....	♂	55	....
27	35 years ago	Hg; KI	♂	58	Ulcer of leg
28	1914	Hg 2 years ago	♂	22	No manifestations
29	1898	....	♂	39	....
30‡	....	606; Hg; KI	♂	36	....
31†	....	606; Hg; KI	♂	36	....
32	....	606; Hg; KI	♂	36	....
33	....	606 (VI); Hg (XV)	♀	33	No manifestations
34	Rash 3 yr. ago; iritis 2 yr. ago	....	♂	30	....
35	....	Hg (XVI)	♀	39	....
36	....	606; Hg; KI	♂	41	....
37	1913	606; Hg; KI	♂	27	Delayed union, fractured tibia
38	....	606; Hg; KI	♀	14	....
39	....	606 (VI)	♀	7	....
40	....	606; Hg	♀	33	No manifestations
41	....	606; Hg; KI	♂	36	....
42‡	....	606; Hg; KI	♂	36	....
43	1912	606 (IV)	♂	34	No manifestations
44	1906	Hg	♂	40	Tubercular syphilid on neck
45	1896	....	♂	44	Ulcerating lesions, cheek and neck
46	1896	Hg	♂	38	....
47	....	606 (III)	♂	31	No manifestations
48	1907	....	♂	35	....
49	1909	606 (III); Hg (XL)	♂	28	....
50	2 months ago	....	♀	19	Sore throat; rash
51	1914	606 (V); Hg (XXX)	♀	30	....
52	....	606(VIII); Hg(XV)	♂	17	Keratitis
53	....	....	♂	32	....
54	....	....	♂	?	....
55	....	....	♂	43	Hydrocele
56†	....	Hg	♂	32	....
57	....	....	♂	27	Penile chancre, 8 days old; spirochetes found
58	1905	....	♂	54	Papular lesions, face
59	....	606; Hg	♀	25	....
60	March, 1916	Hg (XVI); 606 (VI)	♀	29	....
61	1906	....	♂	32	Incontinence
62	8 months ago	Hg (XXXVII)	♂	35	....
63	1912	....	♂	40	....
64	1901	Hg (1 year)	♂	56	Ulcer of nose, 1 year's duration
65	1898	Hg	♂	36	Infected fracture of os calcis; temperature 105
67	....	....	♂	32	....

TABLE 4—*Continued*

BRIEF DATA CONCERNING 92 TESTS ON 80 SYPHILITIC PATIENTS, GROUP I

Diagnosis	Complement-Fixation							
	At 37 C.				At 8 C.			
	1	2	3	4	1	2	3	4
Lues III	—	—	—	—	++++	++++	++++	—
Lues III	—	—	—	—	++++	++++	++++	—
Lues I	—	—	—	—	++++	++++	—	—
Lues III	—	—	++++	—	++++	++++	++++	—
Inactive lues	++	—	—	—	+	+	—	—
Gumma	+++	—	++++	—	++++	++++	++++	—
Lues III	++++	+++	++++	—	++++	++++	++++	—
Lues III	—	—	—	—	—	—	—	—
....	+++	—	—	—	++++	++++	—	—
Gumma	++++	+++	++++	—	++++	++++	++++	—
Lues III	—	—	—	—	+	++++	++++	—
Paresis	—	—	—	—	++++	++++	—	—
Paresis	—	—	—	—	++++	++++	+	—
Paresis	—	—	—	—	—	—	—	—
Inactive lues	++	—	—	—	++++	—	—	—
Inactive lues	++	—	—	—	++++	++++	—	—
....	—	—	—	—	++++	++++	—	—
Lues II	++++	—	++++	—	+	++++	++++	—
....	++++	++++	++++	—	++++	++++	++++	—
....	—	—	—	—	—	—	—	—
....	++++	++	++	—	++++	++++	++++	—
....	++++	+	++++	—	++++	++++	++++	—
....	++++	—	++	—	++++	++++	++++	—
Lues III	++++	++++	++++	—	++++	++++	++++	—
....	—	—	—	—	—	—	—	—
Paresis	++++	++++	++++	—	++++	++++	++++	—
....	—	—	—	—	—	—	—	—
Inactive lues	—	—	—	—	—	—	—	—
Paresis	++++	—	—	—	++++	++++	—	—
Paresis	—	—	—	—	—	—	—	—
Paresis	++++	—	—	—	++++	++++	++++	—
Paresis	++++	—	—	—	++++	+	—	—
....	—	—	—	—	—	—	—	—
....	++++	—	++	—	++++	++++	++++	—
....	++++	—	—	—	++++	++++	++++	—
Paresis	—	—	—	—	++++	++++	++++	—
....	—	—	—	—	++++	++++	—	—
Juvenile paresis	++++	++++	++++	—	++++	++++	++++	—
Congenital lues	++++	++++	++++	—	++++	++++	++++	—
....	—	—	—	—	++++	—	—	—
Paresis	++++	—	—	—	++++	++++	++++	—
Paresis	—	—	—	—	++++	++++	++++	—
....	—	—	—	—	—	—	—	—
Lues III	—	—	—	—	++++	++++	++++	—
Lues III	—	—	—	—	++++	++++	++++	—
....	—	—	—	—	++++	++++	—	—
....	++	—	—	—	++++	—	—	—
....	+	—	—	—	++++	—	—	—
....	++++	—	—	—	++++	++++	—	—
Lues II	++++	++++	++++	—	++++	++++	++++	—
....	—	—	—	—	—	—	—	—
....	—	—	—	—	++++	—	—	—
Congenital lues	—	—	—	—	—	—	—	—
Lues III	—	—	—	—	++++	++++	++++	—
....	++++	++++	++++	—	++++	++++	++++	—
Cerebrospinal lues	—	—	—	—	++++	+	—	—
Lues I	—	—	—	—	—	—	—	—
Lues III	++++	—	—	—	++++	++++	+	—
....	++++	++	++	—	++++	++++	+	—
....	—	—	—	—	—	—	—	—
Tabes	—	—	—	—	++++	+	—	—
....	—	—	—	—	—	—	—	—
....	++++	—	—	—	++++	++++	—	—
Lues III	++++	++++	++++	—	++++	++++	++++	—
....	—	—	—	—	++++	—	—	—
Paresis	++++	++++	++++	—	++++	++++	++++	—

TABLE 4—*Continued*

BRIEF DATA CONCERNING 92 TESTS ON 80 SYPHILITIC PATIENTS, GROUP I

No.	History of Infection	Treatment	Sex	Age	Manifestations at Time of the Tests
68†	....	....	♂	34	....
69‡	....	....	♂	32	....
70	1908	....	♂	47	Papillitis in both eyes....................
70†	....	....	♂	45	A. R. pupils; difficulty in walking; nocturnal micturation
71*	....	606; Hg; KI	♂	36	....
72	1908	....	♂	28	Ulcer of leg................................
73	....	Hg (XVI); 606 (IV)	♂	29	....
74	Husband is luetic	....	♀	19	Cervical and inguinal adenitis............
75	....	Hg; 606	♀	27	....
76	1911	606 (III)	♀	34	....
77	....	....	♀	?	....
78‡	....	606; Hg; KI	♂	36	....
79*	....	606; Hg; KI	♂	41	....
80	....	....	♂	47	....
81‡	....	606; Hg; KI	♂	41	....
82†	....	606; Hg; KI	♂	41	....
83	....	....	♂	50	....
84	....	....	♂	?	....
85	1906	KI (1 year)	♂	52	Joint pains, especially at night..........
86	1913	Hg	♂	39	Dizziness; pain in back..................
87	1900	....	♂	31	Gastric symptoms ....
88*	....	606; Hg; KI	♂	36	....
89	6 weeks ago	....	♂	29	Penile sore; rash; malaise................
90	1906	606 (VI)	♀	30	Generalized body pains....................
91	....	....	♂	33	Swelling of right knee.....................
92	....	....	♂	42	Iritis ....

* A simple number indicates blood serum. A number with asterisk indicates a duplicate specimen of blood serum from a patient previously tested. Specimen 10* was obtained from the same patient as Specimen 6; 71* as 41; 79* as 36; 88* as 30.

† A number followed by this character designates a spinal fluid. The patient's blood serum was usually tested also. The following numbers indicate spinal fluid and blood serum, respectively, from the same patient: 14† and 12; 31† and 32; 69† and 67. Specimens 56†, 68†, and 70† were from patients whose blood serum was not tested.

‡ A number followed by this character designates cerebral fluid. The patient's blood serum was usually tested also. The following numbers indicate cerebral fluid and blood serum, respectively, from the same patient: 30‡ and 32; 42‡ and 41; 69‡ and 67; 78‡ and 71*; 81‡ and 79*.

primary lesion in 1911. The patient was then aged 21 and probably did not, at that time, appreciate the gravity of a little ulcer on the penis. In Case 15 it was stated frankly that the husband was syphilitic. In all of the cases in this group, syphilis was seriously considered in the differential diagnosis and in most of them the tests revealed fixation of complement with all of the antigens, both at 37 C. and 8 C.

At first thought it seems rather inappropriate, in a study of the value of the Wassermann reaction, to utilize the results of this test in the classification of cases. However, this study is not especially concerned with the absolute value of the reaction, but rather with the relative values of different methods of performing it, and we think that we may be allowed the liberty of utilizing the evidence furnished by the Wassermann test along with the other signs and symptoms.

TABLE 4—*Continued*

Brief Data Concerning 92 Tests on 80 Syphilitic Patients, Group I

Diagnosis	Complement-Fixation							
	At 37 C.				At 8 C.			
	1	2	3	4	1	2	3	4
Cerebrospinal lues	—	—	—	—	++++	++++	—	—
Paresis	++++	++++	++++	—	++++	++++	++++	—
....	++++	++	++	—	++++	++++	++++	—
Taboparesis	+	—	—	—	++++	++	—	—
Paresis	—	—	—	—	—	—	—	—
....	++++	++++	++++	—	++++	++++	++++	—
....	++	—	—	—	—	—	—	—
....	++++	++++	++++	—	++++	++++	++++	—
....	++	—	—	—	++++	—	—	—
....	++++	—	—	—	++++	+++	—	—
....	++++	—	—	—	++++	—	++	—
Paresis	—	—	—	—	++++	++++	++++	—
Paresis	—	—	—	—	—	—	—	—
....	++++	++++	++++	—	++++	++++	++++	—
Paresis	—	—	—	—	—	—	—	—
Paresis	—	—	—	—	++++	++++	++++	—
....	++++	++++	++++	—	++++	++++	++++	—
....	+	—	—	—	++++	—	—	—
....	—	—	—	—	—	—	—	—
....	++++	—	—	—	++++	++++	—	—
....	++++	+++	+++	—	++++	++++	++++	—
Paresis	++++	—	—	—	++++	—	—	—
Lues	++++	++++	—	—	++++	++++	++++	—
....	++++	++++	++++	—	++++	++++	++++	—
Charcot's joint	++++	++++	++++	—	++++	++++	++++	—
....	++++	++++	++++	—	++++	++++	++++	—

Table 7 presents, in summarized form, the findings in Group II B. Brief data in regard to each test are given in Table 8.

What has already been said of Tables 3 and 5 may be said, also, of Table 7. Antigen B. H. P., at 8 C., is again quite as good as Antigen B. H. C., at 37 C., but Antigen B. H. C., at 8 C., is the best, yielding 92% positive reactions.

TABLE 5

Summarized Results of 27 Reactions in 24 Patients, Evidently Syphilitic, Group II A

Antigen and Temperature	Positive		Doubtful		Negative	
	Number	Per Cent.	Number	Per Cent.	Number	Per Cent.
1. Cholesterinized at 37 C.	25	92.5	0	0.0	2	7.5
2. Simple at 37 C.	18	66.6	1	3.8	8	29.6
3. Acetone insoluble at 37 C.	21	77.7	0	0.0	6	22.3
4. Cholesterinized at 8 C.	27	100.0	0	0.0	0	0.0
5. Simple at 8 C.	25	92.5	0	0.0	2	7.5
6. Acetone insoluble at 8 C.	24	88.7	1	3.8	2	7.5

For convenience, all of the cases in Groups I, II A, and II B may be considered together as cases certainly or probably of syphilis. The summarized results of these tests, considered together, are shown in Table 9.

TABLE 6

BRIEF DATA CONCERNING 27 TESTS ON 24 PATIENTS EVIDENTLY SYPHILITIC, GROUP II A

No.	Sex	Age	Manifestations at Time of the Tests	Diagnosis
1	♀	21	Eruption on palms of hands......................	Lues III
2	♂	16	Dyspnea; enlarged heart; murmur...............	Aortic regurgitation
3	♀	3½	Pain and disability, right arm (sister luetic).....	Luetic osteoperiostitis
4	♂	29	Mass in nose..	Gumma
5	♂	21	Discharging sinus of jaw............................	Chronic osteomyelitis
6	♂	26	Sore on cheek, 8 months' duration...............	Lues III
7	♂	21	Sore throat..	Lues II
8	♂	1 mo.	Fissures about mouth and anus; malnutrition..	Congenital lues
9	♀	25	Chronic pharyngitis..................................	Lues
10	♀	28	Headache; tumor of frontal bone.................	Gumma
11	♂	29	Lesion on side of nose, 2 months duration.......	Lues
12	♂	36	Lesions on both elbows.............................	Lues
13	♀	55	Chronic arthritis with atrophy of bones of ankle	Lues
14	♂	32	Tumor of tonsil......................................	Gumma
15	♀	34	Neuralgia..	Lues
16	♂	33	Sore throat..	Gumma of larynx
17	♂	11	Acute meningitis.....................................	Meningeal lues
18	♀	23	Sore throat..	Lues II
19	♀	26	Sore throat, 3 weeks' duration....................	Lues II
20*	♀	55	See 13...	....
21†	♂	11	See 17...	....
22	♀	41	Indurated areas, left calf, tending to soften.....	Gummata
23	♂	23	Optic neuritis...	Lues
24	♂	33	Perforation, hard palate............................	Lues
25	♀	28	Lesion on corner of mouth, 1 year old (provocative treatment 1 week ago)	Lues
26	♀	3 mo.	Coryza, loss of weight.............................	Lues
27*	♀	55	See 13...	....

* A simple number indicates blood serum. A number with asterisk indicates a duplicate specimen of blood serum from a patient previously tested. Specimens 20* and 27* were both from the same pattient as 13.

† Specimen 21† was spinal fluid from the same patient who furnished the blood serum Specimen 17.

We come, now, to another group of cases in which again there were no histories of syphilis nor any of antisyphilitic treatment. In some of them the physical findings were rather vague and the clinical diagnoses not stated with any degree of assurance, but in others the findings were quite definitely not those of syphilis and the diagnoses were of conditions not syphilitic, such, for example, as acute articular rheumatism and typhus fever. We shall designate this Group II C. In it there have been placed 16 patients with 16 tests. In all of these there was complete or partial fixation of complement with Antigen B. H. C., either at 37 C. or at 8 C., or both, associated with absence of fixation with either of the other antigens.

The results obtained in this group are summarized in Table 10, and brief data in regard to each test are presented in Table 11.

We feel rather confident that the cases in Group II C were not syphilitic and that the complement-fixation obtained in them was non-

TABLE 6—*Continued*

BRIEF DATA CONCERNING 27 TESTS OF 24 PATIENTS EVIDENTLY SYPHILITIC, GROUP II A

Complement-Fixation							
At 37 C.				At 8 C.			
1	2	3	4	1	2	3	4
++++	+++	+++	—	++++	++++	++++	—
++++	—	++++	—	++++	++++	++++	—
++++	++++	++++	—	++++	++++	++++	—
++++	++++	++++	—	++++	++++	++++	—
++++	—	—	—	++++	++++	++++	—
++++	++++	++++	—	++++	++++	++++	—
++++	++++	++++	—	++++	++++	++++	—
—	—	—	—	++++	—	—	—
++++	++++	++++	—	++++	++++	++++	—
++++	++++	++++	—	++++	++++	++++	—
++++	++++	++++	—	++++	++++	++++	—
++++	—	—	—	++++	—	—	—
++++	++++	++++	—	++++	++++	++++	—
++++	+	++	—	++++	++++	++++	—
++++	—	++	—	++++	++++	++++	—
++++	—	—	—	++++	++++	++++	—
++++	++++	++++	—	++++	++++	++++	—
++	—	—	—	++++	++++	++++	—
++++	++++	++++	—	++++	++++	++++	—
++++	++++	++++	—	++++	++++	++++	—
++++	++++	++++	—	++++	++++	++++	—
++++	++++	++++	—	++++	++++	++++	—
++++	++++	++	—	++++	++++	+	—
—	—	—	—	++++	++++	++++	—
++++	++	++++	—	++++	++++	+++	—
++++	++++	++++	—	++++	++++	++++	—
++++	++++	++++	—	++++	++++	++++	—

TABLE 7

SUMMARIZED RESULTS OF 25 TESTS ON 20 PATIENTS, PROBABLY SYPHILITIC, GROUP II B

GROUPS I, II A, AND II B

Antigen and Temperature	Positive		Doubtful		Negative	
	Number	Per Cent.	Number	Per Cent.	Number	Per Cent.
1. Cholesterinized at 37 C.	21	84.0	0	0.0	4	16.0
2. Simple at 37 C.	12	48.0	2	8.0	11	44.0
3. Acetone insoluble at 37 C.	17	68.0	1	4.0	7	28.0
4. Cholesterinized at 8 C.	23	92.0	1	4.0	1	4.0
5. Simple at 8 C.	22	88.0	2	8.0	1	4.0
6. Acetone insoluble at 8 C.	20	80.0	2	8.0	3	12.0

specific. The cholesterinized antigen, it will be observed, was the only one* to give positive results in this group, and it appeared to be more unreliable at the higher temperature.

There remains to be considered a large group of cases in which there were absolutely no indications of syphilis and which may safely

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* When these results were first compiled, Specimen 1, Table 8, was included in this group. It gave a 2+ reaction with the simple antigen, at 8 C. While this paper was being prepared for publication it was learned that the patient who furnished this specimen of blood was actually a syphilitic. Another specimen from her gave a definite diagnostic Wassermann reaction (Specimen 4, Table 8) and on exploratory laparotomy, the clinical diagnosis of gastric carcinoma was not confirmed.

TABLE 8

Brief Data Concerning 25 Tests on 20 Patients Probably Syphilitic, Group II B

No.	History of Infection	Treatment	Sex	Age	Manifestations at Time of the Tests
1	....	....	♀	32	Constipation ....
2	....	....	♀	23	Pain in left hip and left side; 2 discharging sinuses in back, right side
3	....	....	♀	21	Cutaneous eruption ....
4*	....	....	♀	32	Chronic bowel trouble....................
5	....	....	♂	26	Nervous; tremulous ....
6	....	....	♀	19	Loss of weight; pyloric syndrome.......
7	....	....	♀	19	No manifestations ....
8*	....	....	♀	19	See 6 ....
9	Doubtful, 1911	....	♂	26	No manifestations ....
10	....	....	♂	64	Acute bronchitis; abdominal pain.......
11	....	....	♀	48	Myocarditis; ascites ....
12	....	None	♀	32	Abdominal pain; epigastric tumor......
13*	....	Hg; KI	♀	32	See 12 ....
14	....	....	♀	35	Nervousness ....
15	Husband is luetic	....	♀	22	No manifestations ....
16	....	....	♀	48	Ascites; myocarditis ....
17	....	....	♀	35	Ascites; jaundice ....
18	....	....	♀	42	Hypertrophied turbinate ....
19	....	....	♀	10	Mental retardation ....
20*	....	See 13	♀	32	See 12 and 13............................
21*	....	....	♀	35	See 7 ....
22	....	....	♂	21	Chronic arthritis, elbow.................
23	....	....	♀	58	Headache; facial palsy with anesthesia left side of face; pain in neck
24	....	....	♀	36	Acute bronchitis; myocarditis..........
25	....	....	♀	29	Epilepsy ....

* All specimens in this table were blood serums. Specimen 4* was from the same patient as 1; 8* from the same patient as 6; 13* and 20* from the same patient as 12; 21* from the same patient as 7.

TABLE 9

Summarized Results of 144 Tests on 124 Patients, Certainly or Probably Syphilitic, Groups I, II A, and II B

Antigen and Temperature	Positive		Doubtful		Negative	
	Number	Per Cent.	Number	Per Cent.	Number	Per Cent.
1. Cholesterinized at 37 C........	94	65.3	3	2.1	47	32.6
2. Simple at 37 C.................	54	37.5	4	2.8	86	59.7
3. Acetone insoluble at 37 C......	67	46.5	1	0.7	76	52.8
4. Cholesterinized at 8 C.........	120	83.3	4	2.8	20	13.9
5. Simple at 8 C..................	106	73.6	6	4.2	32	22.2
6. Acetone insoluble at 8 C.......	87	60.4	6	4.2	51	35.4

be termed nonsyphilitics. It is unnecessary to show detailed data in these cases. Suffice it to say that there were 341 tests on 317 nonsyphilitics, in none of which was any degree of fixation obtained with any of the antigens, under either condition of incubation.

The tests shown in Table 10 and the results on nonsyphilitics have been combined in Table 12, which shows the results obtained on the

TABLE 8—*Continued*

BRIEF DATA CONCERNING 25 TESTS ON 20 PATIENTS PROBABLY SYPHILITIC, GROUP II B

Diagnosis	Complement-Fixation							
	At 37 C.				At 8 C.			
	1	2	3	4	1	2	3	4
Carcinoma of stomach	—	—	—	—	—	++	—	—
Tuberculosis or lues	++++	—	—	—	++++	+	—	—
Dermatitis	++++	—	—	—	++++	++++	++	—
Carcinoma of stomach	++++	—	+	—	++++	++++	++	—
....	++++	++++	++++	—	++++	++++	++++	—
Duodenal ulcer	—	—	—	—	++++	+	++++	—
....	++++	—	—	—	++++	++++	+	—
....	—	—	—	—	+	—	++++	—
....	—	—	—	—	++++	++++	—	—
....	++++	+	++	—	++++	++++	++++	—
....	++++	+	+++	—	++++	++++	++++	—
Tumor of pylorus	++++	++++	++++	—	++++	++++	++++	—
See 12	++++	—	++++	—	++++	++++	++++	—
....	++++	++++	++++	—	++++	++++	++++	—
....	++++	++++	++++	—	++++	++++	++++	—
....	++++	—	++	—	++++	++++	+	—
....	++++	++++	++++	—	++++	++++	++++	—
Syphilis	++++	++++	++++	—	++++	++++	++++	—
....	++++	++++	++++	—	++++	++++	++++	—
See 13	++++	++++	++++	—	++++	++++	++++	—
....	++++	++++	++++	—	++++	++++	++++	—
Tubersulosis (?); lues (?)	++++	++++	++++	—	++++	++++	++++	—
....	++++	++++	++++	—	++++	++++	++++	—
....	++++	++++	++++	—	++++	++++	++++	—
....	++++	—	—	—	++++	++++	++++	—

TABLE 10

SUMMARIZED RESULTS OF 16 TESTS ON PATIENTS, PROBABLY NOT SYPHILITIC, GROUP II C

Antigen and Temperature	Positive		Doubtful		Negative	
	Number	Per Cent.	Number	Per Cent.	Number	Per Cent.
1. Cholesterinized at 37 C........	8	50.0	3	18.8	5	31.2
2. Simple at 37 C.................	0	0.0	0	0.0	16	100.0
3. Acetone insoluble at 37 C......	0	0.0	0	0.0	16	100.0
4. Cholesterinized at 8 C.........	5	31.2	3	18.8	8	50.0
5 Simple at 8 C..................	0	0.0	0	0.0	16	100.0
6. Acetone insoluble at 8 C.......	0	0.0	0	0.0	16	100.0

357 specimens which we consider to be nonsyphilitic. One might obtain an exaggerated idea of the unreliability of the cholesterinized antigen if he regarded only the percentages shown in Table 10. From Table 12 it would appear that this antigen has given a positive result in 2.2% of tests on nonsyphilitic specimens, at 37 C., and in 1.4% of tests on such specimens at the lower incubation temperature, in this series.

TABLE 11

BRIEF DATA CONCERNING 16 TESTS ON 16 PATIENTS, PROBABLY NOT SYPHILITIC, GROUP II C

No.	History of Infection	Sex	Age	Manifestations at Time of the Tests	Diagnosis
1	....	♂	30	Enlarged liver; jaundice................	Hepatic cirrhosis
2	....	♀	22	Acute arthritis..........................	Acute articular rheumatism
3	....	♂	40	Enlarged liver; ascites, jaundice.......	Hepatic cirrhosis
4	....	♀	26	Pain in lumbar region; loss of weight..	....
5	....	♂	20	....	Hysteria
6	....	♀	6	....	Cretin
7	....	♂	24	Fever, headache, backache, eruption...	Typhus fever
8	....	♀	37	Abdominal pain.........................	Duodenal ulcer
9	....	♀	25	Nervousness............................	Endocervicitis
10	....	♀	3	Mental deficiency.......................	....
11	....	♀	23	Jaundice................................	Catarrhal cholangitis
12	....	♀	8½	....	Miliary tuberculosis
13	Father said to be luetic	♀	2½	....	Idiocy
14	Two babies died under 5 days	♀	25	....	....
15	....	♂	20	Abscess, prepatellar bursa.............	Secondary to wound of leg
16	....	♀	30	Cervical adenitis........................	Tuberculosis (?); lues (?)

TABLE 12

SUMMARIZED RESULTS OF 357 TESTS ON 333 NONSYPHILITIC PATIENTS, GROUPS II C AND III

Antigen and Temperature	Positive		Doubtful		Negative	
	Number	Per Cent.	Number	Per Cent.	Number	Per Cent.
1. Cholesterinized at 37 C.........	8	2.2	3	0.8	346	97.0
2. Simple at 37 C..................	0	0.0	0	0.0	357	100.0
3. Acetone insoluble at 37 C......	0	0.0	0	0.0	357	100.0
4. Cholesterinized at 8 C..........	5	1.4	3	0.8	349	97.8
5. Simple at 8 C...................	0	0.0	0	0.0	357	100.0
6. Acetone incoluble at 8 C.......	0	0.0	0	0.0	357	100.0

SUMMARY

Wassermann tests have been performed on 501 identical specimens from 457 cases by 6 different methods. The cases have been divided into 2 classes, on the basis of the histories, those giving histories of syphilis and those not giving such histories. The 1st class was called Group I. The 2nd class was subdivided into several groups by a consideration of the symptoms and signs, including the Wassermann reaction. Groups II A and II B are composed of cases which presented symptoms and signs of syphilis, more definite in II A than in II B. Both of these subgroups are considered as probably luetic. Group II C comprises cases in which the only evidence of syphilis was

TABLE 11—*Continued*

BRIEF DATA CONCERNING 16 TESTS ON 16 PATIENTS, PROBABLY NOT SYPHILITIC, GROUP II C

Complement-Fixation							
At 37 C.				At 8 C.			
1	2	3	4	1	2	3	4
++	−	−	−	++	−	−	−
++	−	−	−	+	−	−	−
−	−	−	−	++++	−	−	−
−	−	−	−	+	−	−	−
−	−	−	−	+	−	−	−
+	−	−	−	−	−	−	−
++++	−	−	−	−	−	−	−
+++	−	−	−	−	−	−	−
++	−	−	−	−	−	−	−
+	−	−	−	−	−	−	−
++++	−	−	−	−	−	−	−
+	−	−	−	−	−	−	−
++++	−	−	−	++++	−	−	−
−	−	−	−	++++	−	−	−
++++	−	−	−	−	−	−	−
−	−	−	−	++++	−	−	−

complement-fixation with 1 antigen. These cases are considered as probably not syphilitic. Group III is made up of the rest of the cases, in which there was no evidence of syphilis.

The 6 methods consisted in the use of 3 different antigens, namely, alcoholic extract of beef heart, cholesterinized (Antigen B. H. C.), plain alcoholic extract of beef heart (Antigen B. H. P.) and acetone insoluble lipoid antigen prepared from the same extract of beef heart (Antigen B. H. A.), under 2 sets of incubation conditions, namely, at 37 C., for 1 hour, and at 8 C., for 4 hours.

It was found that in cases of syphilis and in cases probably syphilitic, the highest percentage of positive reactions was obtained in Method 4, that is, with cholesterinized antigen (B. H. C.), at 8 C., for 4 hours. The other methods, in order of value, were plain antigen (B. H. P.), at 8 C., cholesterinized antigen (B. H. C.), at 37 C., acetone insoluble antigen (B. H. A.), at 8 C., B. H. A., at 37 C., and B. H. P. at 37 C.

Complement-fixation was obtained in a small number of cases which were probably not syphilitic. In all of these cases, the antigen which yielded the fixation was Antigen B. H. C., sometimes at 37 C., sometimes at 8 C., and in a few instances both at 37 C. and at 8 C.

A comparatively large number of negative controls were tested, in none of which, with the exception of the few cited above, which have been considered in a separate group, was any complement-fixation obtained.

CONCLUSIONS

1. The use of the cholesterinized antigen, with the first incubation, at 8 C., for 4 hours, constitutes a more sensitive test for syphilis than does any of the other methods examined.
2. The cholesterinized antigen, both at 37 C. and at 8 C., is apt to yield nonspecific complement-fixation. Therefore, in a diagnostic reaction, fixation with the cholesterinized antigen alone is, at best, of only doubtful significance.
3. The simple extract antigen, with the first incubation at 8 C., is more sensitive than the cholesterinized antigen at 37 C., and in this series it did not give any false positive reactions, according to the available evidence.
4. The acetone insoluble preparation, made according to the method of Noguchi, is less sensitive, either at 37 C., or at 8 C., than is the cholesterin-reinforced antigen at either temperature, and is also less sensitive than the simple extract at 8 C. It is more sensitive than the simple extract at 37 C. and, in this series, has, according to the available evidence, given no false positive reactions.